

I. AMENDMENTS TO THE CLAIMS

This listing of the claims will replace all prior versions and listings. Claims 1-7 are currently pending in this application.

LISTING OF CLAIMS:

Claim 1 (previously presented): A method of assembling PCR fragments, comprising

- a) making a first PCR fragment with first and second primers, wherein the second primer comprises a modified nucleotide that can be removed by a DNA repair enzyme, resulting in a 3' overhang, and wherein the first PCR fragment comprises a first site specific recombinase site;
- b) treating the first PCR fragment with a DNA repair enzyme to generate a 3' overhang and immobilizing the first PCR fragment on a solid support or vice versa;
- c) making a second PCR fragment with third and fourth primers, wherein the third and fourth primers each comprises a modified nucleotide that can be removed by a DNA repair enzyme resulting in a 3' overhang;
- d) treating the second PCR fragment with a DNA repair enzyme to generate a 3' overhang;
- e) annealing and ligating the first and second PCR fragments;
- f) optionally repeating steps c, d and e until a last PCR fragment is added to the growing chain to produce an assembled fragment, wherein the last PCR fragment comprises a second site specific recombinase site; and
- g) simultaneously removing and circularizing the assembled fragment from the solid support with a site specific recombinase in a single step.

Claim 2 (original): The method of claim 1, where one of the PCR fragments comprises an origin of replication and a selectable marker.

Claim 3 (original): The method of claim 1, wherein the first PCR fragment or the last PCR fragment comprises an origin of replication and a selectable marker.

Claim 4 (original): The method of claim 1, wherein the site specific recombinase is CRE and the site specific recombinase site is lox.

Claim 5 (original): The method of claim 1, wherein the nucleotide is deoxyuridine and the DNA repair enzyme is uracil-DNA-glycosylase followed by T4 endonuclease V.

Claim 6 (original): The method of claim 5, wherein the assembled DNA is greater than 30 kb.

Claim 7 (original): The method of claim 5, wherein the assembled DNA is greater than 30, 40, 50, 75, 100, 125, 150, 200, 250, 300, 350, 400, 450, 500, 750, 1000 or 1500 kb.